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CAMPBELL FILE

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361
OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

OCT 17 1991

MEMORANDUM

SUBJECT: Additional Data for 2,4-DB Mouse Carcinogenicity Study

TO: Ms. J. Coombs, PM 71
SRRD (H7508W)

FROM: Byron T. Backus, Ph.D., Toxicologist Byron T. Backus 8/29/91
Toxicology Branch II
HED (H7509C)

THROUGH: K. Clark Swentzel K. Clark Swentzel 7/30/91
Section Head, Review Section II
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and

Marcia van Gemert, Ph.D., Branch Chief Marcia van Gemert 9/6/91
Toxicology Branch II
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DP Barcode: D166574

Project No. 1-1825 MRID 419362-01

Tox. Chem. 316

Action Requested:

Review additional data (including historical control incidences for hepatocellular adenomas and carcinomas in male mice, as well as incidences for bronchiolar and alveolar adenomas and carcinomas in male mice, and a justification for the highest dose - 750 ppm - selected for female mice) relating to a mouse carcinogenicity study performed at Hazleton Laboratories.

Background:

A lifetime (78-week) mouse carcinogenicity study on 2,4-DB Acid was previously reviewed (DER X, copy attached, as part of a memorandum dated January 20, 1988) in the 2,4-DB Toxicology Chapter of the Registration Standard. At that time it was stated that there was weak, but possible dose-relationship trend involving hepatocellular

carcinoma in males which needed to be addressed with historical control data. In addition, it was noted that justification should be made for 750 ppm in females as a MTD, or being reasonably close to such a level.

Comments and Recommendations:

1. The registrant has submitted historical control data from 8 Hazelton (Wisconsin) mouse studies for hepatocellular carcinomas and adenomas, as well as alveolar carcinomas and alveolar/bronchiolar adenomas. Of these 8 studies, one (H(CB)C4 1987) appears to be control data from the 2,4-DB study. Based on historical control data from the performing laboratory, it is concluded that there is no indication that dietary exposure to the 2,4-DB acid causes any increased incidence of tumors in male mice, and the registrant has sufficiently addressed this point.

2. To justify 750 ppm as a MTD for females, the registrant has cited body weight gain data from a preliminary 4-week range-finding study, in which females at 300 and 1000 ppm had weight gains that were respectively 18% and 15% lower than those of controls during the four week study. However, within the 78-week study, there was very little difference in weight gains between different groups. Interpretation of data from the 78-week study is also made somewhat difficult by the fact that the mean weight for high-dose (750 ppm) females at week 0 was significantly ($p \leq 0.01$) lower than that for controls (20.1 vs. 21.0 gm), while at one week values were 21.8 and 22.5 gm respectively ($p \leq 0.05$). For males, there never was any consistent significant difference between controls and high-dose (750 ppm) mice with respect to mean body weights, despite an increased mortality in 750 ppm mice that necessitated early sacrifice (at 66 weeks) in this group. Further, the comment in the original report (Vol. I, p. 14) was that "the probable maximum tolerated dose for an oncogenicity study, based on increased liver weight, was less than 1,000 ppm." The material in MRID 419362-01 does not mention increased liver weight. It is concluded then that insufficient information has been given to justify 750 ppm as a MTD in females; the registrant should submit information - particularly relating to liver weight findings in the 4-week range-finding study - that more specifically address this point.

3. Without information adequately demonstrating that 750 ppm is a MTD (or is sufficiently close to a MTD) the study remains classified as core supplementary data. This study does not currently satisfy the guideline data requirements [83-2(b)] for a mouse carcinogenicity study.

4. A copy of this memorandum should be made available to the registrant.

Discussion:

According to the original review (memorandum dated January 20, 1988): "There is a weak, but possible dose-relationship trend involving hepatocellular carcinomas in males, with overall group incidences of 0/70. 1/70, 4/69 and 3/70 (the last group is from 750 ppm males, terminated about 3 months before others). The p (by Fisher's exact test) associated with the overall incidence of this tumor type in 250 ppm males (4/69) versus controls (0/70) is 0.058, a close approach to statistical significance. While these findings are certainly not convincing evidence of oncogenicity (it is noted that p obtained by comparing incidences of combined hepatocellular adenomas and carcinomas for 250 ppm males - 7/69 - and controls - 4/70 - is 0.2576), the situation has to be addressed... Some information...on this point is appropriate, particularly as there were no occurrences of bronchiolar adenomas and carcinomas, or of hepatocellular carcinomas in the male mice of this study, although these tumors did occur in male groups which were exposed to the 2,4-DB."

The registrant has submitted historical control data from 8 Hazelton (Wisconsin) mouse studies. Of these 8 studies, one (H(CB)C4 1987) appears to be control data from the 2,4-DB study; cumulative incidences for hepatocellular carcinoma and adenoma are reported as 15/537 and 29/537 respectively, or 0.0279 and 0.054; without findings from the 2,4-DB study these values are 15/467 and 25/467. By Fisher's Exact Test the p value associated in comparing 0/70 and 15/467 is 0.1194, and that with 4/70 and 25/467 is 0.5377. Higher incidences of hepatocellular carcinoma (12/222) were observed in the three studies run for 96-104 weeks, then in the five studies run for 78 weeks (3/315). In addition to the putative 2,4-DB study, there were two 78-week studies (H(CD)C6, 1987, and H(CD)C7, 1989) in which no hepatocellular carcinomas were observed in control males (incidences of 0/70 and 0/65 respectively). In the 2,4-DB mouse study, the incidence of hepatocellular carcinomas in 250 ppm males was 4/69; comparing 4/69 with 15/467 by Fisher's Exact Test gives p = 0.2194.

For alveolar carcinomas and alveolar/bronchiolar adenomas, cumulative control incidences from the eight studies are reported as 26/538 and 16/538 respectively; without data from the 2,4-DB study incidences were 26/468 and 16/468. The p values associated in comparing 0/70 with 26/468 and 0/70 with 16/468 are 0.0243 and 0.1039. However, incidences for alveolar carcinoma in males varied widely in the other studies, ranging from 0/50 to 11/64; higher incidences were associated with longer study durations (96-104 wweks). In the four other studies run for 78 weeks only one or fewer males (out of at least 50) was observed to have this finding; consistent with incidences in 2,4-DB exposed males (25 ppm: 1/70; 250 ppm: 1/70; 750 ppm: 0/70). It is then concluded that, based

on historical control data from the performing laboratory, there is no indication that dietary exposure to the test material causes any increased incidence of tumors in male mice, and the registrant has sufficiently addressed this point.

To justify 750 ppm as a MTD for females, the registrant has cited body weight gain data from a preliminary 4-week range-finding study. The following table is presented (p. 5):

BODY WEIGHT GAINS IN GRAMS (AND PERCENT DIFFERENCE FROM CONTROLS)

		2,4-DB Technical (ppm)				
		0 ppm	30 ppm	100 ppm	300 ppm	1000 ppm
Male	Mean	9.9	9.5	9.3(6%)	8.4(15%)	8.2(17%)
	N	10	10	10	10	10
Female	Mean	5.4	6.2	5.3	4.4(18%)	4.6(15%)
	N	10	10	10	10	10

"Based on these data, it was felt that a dose of 1000 ppm or more would exceed the MTD when administered to the mouse for a lifetime study. The concern at a dose level of 1000 ppm was supported by the 15-18% decrease in body weight gain at 300 ppm in this range-finding study. Therefore, the level of 750 ppm, a reduction from 1000 ppm, was chosen as the high dose for the oncogenicity study."

Within the 78-week study, there was very little difference in weight gains between different groups. However, interpretation of the data is made somewhat difficult by the fact that the mean weight for high-dose (750 ppm) females at week 0 was significantly ($p \leq 0.01$) lower than that for controls (20.1 vs. 21.0 gm); at one week the values were 21.8 and 22.5 gm respectively. For males there never appeared to be a significant difference in mean weights between controls and 750 ppm animals, despite the increased mortality in 750 ppm mice that resulted in their sacrifice at week 66.

In addition, the justification given in this additional material that a MTD had been reached does not correspond to that in the original study. From vol. I, p. 14: "Dose levels for this study were selected based on results from a 4-week range-finding study (HLA Study No. 6158-102). Mice received 2,4-DB in the diet at levels of 0, 300, 1,000 or 3,000 ppm. The data indicated that the probable maximum-tolerated dose for an oncogenicity study, <u>based on increased liver weight</u> (emphasis added by this reviewer), was less than 1,000 ppm." It is concluded then that insufficient information has been given to justify 750 ppm as a MTD in females; the registrant should submit information - particularly relating to liver weight findings in the 4-week range-finding study - that more specifically address this point. Without information adequately demonstrating that 750 ppm is a MTD (or is sufficiently close to a MTD) the study remains classified as core supplementary data.

CASWELL FILE

Reviewed by: Byron T. Backus Byron T. Backus 12/09/87
Section 3, Tox. Branch (TS-769C)
Secondary reviewer: Marcia van Gemert M. van Gemert 12/10/87
Section 3, Tox. Branch (TS-769C)

DATA EVALUATION REPORT X

STUDY TYPE: Lifetime (78 week) dietary & oncogenicity study - mouse

TOX. CHEM. NO: 316

ACCESSION NUMBER: not given

MRID NO: not given

TEST MATERIAL: 2,4-DB

SYNONYMS: 4-(2,4-dichlorophenoxy)butyric acid

STUDY NUMBER(S): HLA 6158-104

SPONSOR: 2,4-DB Task Force

TESTING FACILITY: Hazleton Laboratories, America Inc.

TITLE OF REPORT: Lifetime Dietary Oncogenicity Study in Albino Mice with 2,4-DB

AUTHOR(S): MacKenzie, Karen M.

REPORT ISSUED: 6/23/87

Classification: core supplementary data (additional information and clarifications are needed as indicated below to upgrade the classification of this study).

Special Review Criteria (40 CFR 154.7)

CONCLUSIONS:

1. Groups (50 of each sex) of Crl:CD®1(CR)BR mice/~~sex~~ were fed 2,4-DB in their diet at 0, 25, 250 and 750 ppm (equivalent to 0, 3.75, 37.5 and 112.5 mg/kg/day) for 78 weeks (except for males at 750 ppm, which were terminated at 66 weeks). An additional 20 animals/sex/dosage group were terminated after having received these doses in their diet for 52 weeks.

2. There is a weak, but possible dose-relationship trend involving hepatocellular carcinomas in males, with overall group incidences of 0/70, 1/70, 4/69 and 3/70 (the last value is from 750 ppm males, terminated about 3 months before others). The p (by Fisher's exact test) associated with the overall incidence of this tumor type in 250 ppm males (4/69) versus controls (0/70) is 0.058, a close approach to statistical significance. While these findings are certainly not convincing evidence of oncogenicity (it is noted that p obtained by comparing incidences of combined hepatocellular adenomas and carcinomas for 250 ppm males - 7/69 - and controls - 4/70 - is 0.2576), the situation has to be addressed. Although the laboratory has indicated a reluctance to submit historical control data on this mouse strain, some information (perhaps from the most recent series

of studies conducted in support of pesticide registrations) on this point is appropriate, particularly as there were no occurrences of bronchiolar adenomas and carcinomas, or of hepatocellular carcinomas in the control males of this study, although these tumors did occur in male groups which were exposed to the 2,4-DB.

2. The high incidence of mortalities which occurred starting about week 58 in 750 ppm males (and necessitating the termination of the remaining animals in this group at week 66) is conclusive evidence that these animals were dosed at (or even somewhat above) a maximally tolerated dose level.

3. With the females there is no evidence of any dose-related trend in bronchiolar, liver or any other types of tumors, and the incidences of neoplasms in females in this study were quite low for all groups. However, the major question is whether in fact the females were tested at a maximally tolerated dose (MTD) or close enough to such a level, particularly as the only major effect in 750 ppm females was a significant increase in mean kidney weights.

   Justification should be made then that 750 ppm in females is a MTD (or is close enough to such a level). Information submitted should include data, preferably for both sexes, from the 4-week range-finding study (HLA No. 6158-102) used in setting the dose levels of this oncogenicity study.

A. MATERIALS:

1. Test compound: 2,4-DB Technical, Description: a white granular substance. No batch number given. Purity 97.74% (see vol. II, p. 420). Material used was received April 3, 1984, and was stored frozen until June 16, 1984. It was then ground and "placed into 83 glass quart jars with Teflon®-lined lids...and stored frozen under nitrogen through the study."

2. Test animals: Species: mouse; Strain: Crl:CD®1(CR)BR; "purchased as weanlings...animals were received on September 25, 1984, and...were acclimated for 16 days before being placed on test." Treatment was initiated when the animals were approximately 5 weeks old.

B. STUDY DESIGN:

1. Dose levels

   From vol. I, p. 14: "Dose levels for this study were selected based on results from a 4-week range-finding study (HLA Study No. 6158-102). Mice received 2,4-DB in the diet at levels of 0, 300, 1,000 or 3,000 ppm. The data indicated that the probable maximum-tolerated dose for an oncogenicity study, based on increased liver weight, was less than 1,000 ppm. The following levels were selected for the long-term study: 0, 25, 250 or 750 ppm 2,4-DB."

2. Animal assignment

Animals were randomly assigned to groups as indicated below:

Test Group	Dose in diet (ppm)	Oncogenicity		Interim Sac. 12 months		Others	
		male	female	male	female	male	female
1 Control	0	50	50	20	20	-	-
2 Low	25	50	50	20	20	-	-
3 Mid	250	50	50	20	20	-	-
4 High	750	50	50	20	20	-	-
Prestudy data	0	-	-	-	-	10	10
Sentinel animals	0	-	-	-	-	-	-

3. Diet preparation

Appropriate amounts of 2,4-DB were mixed, on a weekly basis, with Purina Certified Rodent Chow® 5002. Prepared diets were refrigerated until fed to the mice. From vol. I p. 17: "Homogeneity determinations were done on all dose levels. Samples were collected from the top, two opposing sides, and bottom of the mixing bowl and analyzed. Stability of 2,4-DB in the diet was determined by analyzing samples of the low- and high-dose diets stored at room temperature for 0, 7, 14, 21 and 28 days."

"Duplicate samples of all diets mixed for Weeks 1 through 4 were analyzed. Subsequently, one dose level was selected each week for assay and frozen until analysis."

Results - The following sample analyses are among those reported (vol. 1, p. 46-52):

Nominal level (ppm)	Analytical Results (ppm)				
	week 1	week 4	wk 20-22	wk 43-45	week 78
25	26.8	26.2†	23.9†	27.3†	24.2†
250	246	249†	241†	233†	239†
750	748	730†	709†	734†	721†

†Mean of 2 or more measurements.

The range in analyses at 25 ppm was from 19.8 to 34.2 ppm; for 250 ppm it was 178 to 265 ppm, and for 750 ppm it was usually from 529 to 778 ppm. However, for week 26 for the nominally 750 ppm sample values ranged from less than 10 ppm to 172 ppm (see vol. I, p. 48). The section on deviations from protocol (vol. I, p. 228-230) does not indicate any mis-mixing occurred at this time (or any other), and the text comment (vol. I, p. 20) is: "Week 26 samples were sent to the Sponsor for analysis; results for the 25-, 250- and 750-ppm diets were 25.6, 229 and 511 ppm, respectively."

4. Animals received food (according to the protocol in vol. I, p. 239 food was to be made available "from glass jars that limit spillage and allow easy inspection of amount and condition of feed.") and water ad libitum.

5. Statistics - From vol. I, p. 36: "Various models of calculators, computers, and computer programs were used to analyze data in this study." From appendix M, volume V, p. 1589: "Standard one-way analysis of variance (ANOVA) was used to analyze the following data for each sex: body weight; body weight gain; food consumption; clinical chemistry and hematology (except erythrocyte morphology); organ weight; organ-to-body weight percentages; and organ-to-brain weight ratios." For survival and tumor analyses "cumulative survival data were analyzed using the National Cancer Institute package. Trend analysis of survival is evaluated at the 5.0% one-tailed probability level." Both the Cochran-Armitage test for trend and Fisher's exact method for group differences were used to compare the distribution of certain neoplastic and associated hyperplastic lesions.

6. A signed Quality assurance statement is provided on p. 4 of vol. I. A listing of the dates of quality assurance inspections is provided on p. 5 and 6.

C. METHODS AND RESULTS:

1. Observations

From vol. I, p. 239 animals were to be "observed at least twice daily (a.m. and p.m.) for moribundity, death and/or other obvious signs of toxicity." Mice were removed from their cages and carefully examined once a week.

Toxicity/Mortality (survival)

High-dose (750 ppm) males (but not females) had a dramatic increase in mortality commencing at about weeks 58-61 as compared with controls and the two lower dose groups. As a result, all 750 ppm males were terminally sacrificed at week 66; the remaining groups were not sacrificed until week 78.

The following survival data are from table 4 (vol I, p. 57-60):

	Male groups (2,4-DB in ppm)				Female groups (2,4-DB in ppm)			
Week	0	25	250	750	0	25	250	750
1	70/70	70/70	70/70	70/70	70/70	70/70	69/70	70/70
13	70/70	70/70	70/70	70/70	68/69	70/70	67/69	70/70
26	69/70	70/70	70/70	69/70	67/69	70/70	65/68	69/70
52	64/70	69/70	67/70	64/70	61/69	65/70	63/67	66/70
53[a]	44/53	50/51	47/51	44/51	44/52	45/50	45/49	46/50
59	41/53	48/51	42/51	36/51	44/52	44/50	43/49	43/50
61	40/53	45/51	41/51	30/51	44/52	44/50	43/49	43/50
66	38/52	39/51	38/51	20/51[b]	44/52	41/50	42/49	43/50
78	25/52	27/51	25/51	-	39/52	34/50	37/49	40/50

a = after interim sacrifice; b = 750 ppm males sacrificed at week 66.

2. Body weight: Animals were weighed weekly through the first 14 weeks of the study, and every 4 weeks thereafter (it is noted that mice were weighed at week 24, rather than 22).

Representative mean weights: from tables 7 and 8):

	Male groups (2,4-DB in ppm)				Female groups (2,4-DB in ppm)			
Week	0	25	250	750	0	25	250	750
0	24.0	24.1	23.9	23.8	21.0	20.3*	20.5	20.1**
1	26.4	26.3	26.3	26.4	22.5	21.9*	21.9*	21.8*
13	34.2	34.5	34.9	34.2	28.2	27.1**	28.0	27.5
26	36.3	36.4	37.2	36.4	30.6	30.2	31.0	29.9
54	37.3	37.9	39.0	37.6	33.0	33.5	33.5	32.6
66	38.1	38.4	39.4	38.1	34.8	34.7	34.8	33.6
78	37.1	38.3	38.5	†	34.5	33.8	34.4	33.3

* significantly different from control value, $p \leq 0.05$
** significantly different from control value, $p \leq 0.01$
† all 750 ppm males were sacrificed at week 66.

Cumulative mean weight gains:

During the period from week 14 through week 58 males at 250 ppm sporadically had significantly higher cumulative mean weight gains than their controls ($p \leq 0.01$ at weeks 30 and 50), as did females at 250 ppm for weeks 9, 10 and 14 ($p \leq 0.01$ for weeks 9 and 14).

3. Food consumption and compound intake

Consumption was determined on a weekly basis through the first 14 weeks of the study, and every 4 weeks thereafter.

None of the weekly mean food consumption values for any of the male or female groups exposed to the 2,4-DB were significantly lower than corresponding control levels. However, on several occasions, they were significantly higher.

Mean compound consumption data (in mg/kg/day) are given in tables 13 (males) and 14 (females). Mean values for 750 ppm males ranged from 78.33 (week 58) to 171.09 (week 1); for 750 ppm females the range was from 83.39 (week 78) to 214.35 (week 5).

4. Ophthalmalogical examination

All eyes were examined ophthalmalogically before the study was initiated, and then again near termination.

Results: From vol. I, p. 21: "Slight increases in the occurrence of cataract, phthisis bulbi, and retinal degeneration for males or females in the 750-ppm dose group were not considered to be treatment related because the changes were unilateral."

From table 6 (vol. I, p. 78) retinal degeneration was observed only in the eyes of 750 ppm males. The following numbers of

occurrences for each group are reported:

Retinal degeneration - males only:

	0 ppm	25 ppm	250 ppm	750 ppm
Both eyes	-	-	-	1
Right eye	-	-	-	4
Left eye	-	-	-	1

There were probably considerably fewer males in the 750 ppm group (20 at week 66 when they were terminated) than the others when this examination was made. However, appendix E only indicates which animals showed ophthalmic abnormalities, not how many were examined.

5. Blood was collected from the orbital plexus of each animal scheduled for sacrifice at 12 months. The CHECKED (X) parameters were examined.

a. Hematology

X		X	
X	Hematocrit (HCT)		Leukocyte differential count
X	Hemoglobin (HGB)	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)	X	Mean corpuscular HGB conc. (MCHC)
X	Erythrocyte count (RBC)	X	Mean corpuscular volume (MCV)

Blood smears were made from all mice at 12 months and at study termination (week 66 for 750 ppm males and week 78 for other groups). At termination, differential blood counts were made for all male groups and for control and 750 ppm females.

Results: At 12 months: "Males given 25, 250, or 750 of the test material had slightly higher red blood cell count, hemoglobin and hematocrit...differences were significant for hemoglobin in males given 25 or 250 ppm and for hematocrit in males given 250 ppm. Males given 750 ppm had significantly higher relative neutrophil count and significantly lower relative lymphocyte count. Males and females given 750 ppm had significantly lower relative eosinophil counts"

Males only:

	0 ppm	25 ppm	250 ppm	750 ppm
RBC count ($10^6/mm^3$)	8.40	9.14	9.23	8.93
Hemoglobin (HGB)	14.0	15.2*	15.4*	14.8
Hematocrit	44.3	47.9	49.3*	47.0
Differential				
% neutrophils	34	36	39	46**
% lymphocytes	64	62	59	52**
% eosinophils	1	1	2	0**
Females				
Differential				
% eosinophils	2	not done	not done	1**

* Significantly different from control value at $p \leq 0.05$
**Significantly different from control value at $p \leq 0.01$

b. Clinical Chemistry

The CHECKED (X) parameters were examined in animals sacrificed at 12 months:

X	Enzymes:	X	Other:
X	Alkaline phosphatase	X	Total Bilirubin
X	Sorbitol dehydrogenase	X	Blood urea nitrogen
X	Gamma glutamyl transferase	X	Total Cholesterol
X	Serum alanine aminotransferase (ALT; also SGPT)		
X	Serum aspartate aminotransferase (also SGOT)		

Results:

At 12 months: "...Sorbitol dehydrogenase was significantly higher in males given 750 ppm."

Males at 12 months:

	0 ppm	25 ppm	250 ppm	750 ppm
Sorbitol DH (IU/L)	21.6	25.6	34.2	33.8*
Number of animals	10	6	4	6

* Significantly different from control value at $p \leq 0.05$

6. Urinalysis

Urinalysis was not done.

7. Sacrifice and Pathology

All animals that died and that were sacrificed on schedule (at 12 months and at termination) were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs in addition were weighed.

X	Digestive system	X	Cardiovasc./Hemat.	X	Neurologic
	Tongue	X	.Aorta*	XX	.Brain*†
X	.Salivary glands*	XX	.Heart*	X	.Periph. nerve*
X	.Esophagus*	X	.Bone marrow*	X	.Spinal cord (3 levels)*
X	.Stomach*	X	.Lymph nodes*	X	.Pituitary*
X	.Duodenum*	XX	.Spleen*	X	Eyes*
X	.Jejunum*	X	.Thymus*		Glandular
X	.Ileum*		Urogenital	XX	.Adrenals*
X	.Cecum*	XX	.Kidneys*†	X	.Exorbital Lacrimal gland
X	.Colon*	X	.Urinary bladder*	X	.Mammary gland & skin*
X	.Rectum*	XX	.Testes*†	X	.Parathyroids*
XX	.Liver*†	X	.Epididymides	X	.Thyroids*
X	Gall bladder*	X	.Prostate		Other
X	.Pancreas*	X	.Seminal vesicle		.Bone*
	Respiratory	XX	.Ovaries*†	X	.Skeletal muscle*
X	.Trachea*	X	.Uterus*	X	.Skin*
X	.Lungs*	X	.Cervix	X	.All gross lesions*
		X	.Vagina		

* Required for subchronic and chronic studies
† Organ weights required in subchronic and chronic studies

Results:

a. Organ weights

At 12 months and at termination mean kidney weights (absolute and relative to body weight) were higher in both sexes at 750 ppm, usually significantly so. Mean liver weights in the 750 ppm mice tended to be elevated, but not significantly so:

Mean organ wts (gms) - males at 12 months:

	0 ppm	25 ppm	250 ppm	750 ppm
left kidney	0.345	0.340	0.358	0.385
right kidney	0.346	0.344	0.372	0.396
Liver	1.637	1.649	1.691	1.749

12-month mean organ-to-body weight ratios in males (in percent):

	0 ppm	25 ppm	250 ppm	750 ppm
left kidney	1.0340	1.0111	1.0137	1.1957*
right kidney	1.0334	1.0199	1.0539	1.2313*
Liver	4.9280	4.9320	4.7741	5.4447

Mean organ wts (gms) - females at 12 months:

	0 ppm	25 ppm	250 ppm	750 ppm
left kidney	0.235	0.246	0.248	0.276**
right kidney	0.235	0.250	0.252	0.281**
Liver	1.379	1.516	1.502	1.678

12-month mean organ-to-body weight ratios in females (in percent):

	0 ppm	25 ppm	250 ppm	750 ppm
left kidney	0.8049	0.8700	0.8210	0.9516**
right kidney	0.8057	0.8870	0.8351	0.9691**
Liver	4.6869	5.3742	4.9416	5.7919

* Significantly different from control value at $p \leq 0.05$
** Significantly different from control value at $p \leq 0.01$

Significant differences and/or noticeable elevations in organ weights for 750 ppm mice at termination were noted in the following:

Mean organ wts (gms) - males at termination:

	0 ppm	25 ppm	250 ppm	750 ppm
heart	0.225	0.248	0.227	0.256*
left kidney	0.370	0.403	0.416	0.421
right kidney	0.380	0.418	0.420	0.448**
Liver	2.097	2.316	2.260	2.385

Mean organ-to-body weight ratios (in percent) in males at termination:

	0 ppm	25 ppm	250 ppm	750 ppm
heart	0.6189	0.6657	0.6165	0.6700
left kidney	1.0101	1.0767	1.1272	1.1070
right kidney	1.0382	1.1161	1.1396	1.1810
Liver	5.7293	6.2201	6.1507	6.2427

Mean organ wts (gms) - females at termination:

	0 ppm	25 ppm	250 ppm	750 ppm
left kidney	0.284	0.269	0.280	0.308*
right kidney	0.281	0.274	0.295	0.311**

Mean organ-to-body weight ratios (in percent) in females at termination:

	0 ppm	25 ppm	250 ppm	750 ppm
left kidney	0.8384	0.8109	0.8234	0.9448**
right kidney	0.8286	0.8223	0.8672	0.9536**

* Significantly different from control value at $p \leq 0.05$
** Significantly different from control value at $p \leq 0.01$

b. Gross pathology

The 750 ppm males which died or were sacrificed moribund had an increased incidence of staining in the perineal/perianal region. There were also increased incidences of irregularly shaped kidneys and diffusely darkened livers (the latter finding was also more frequent in 750 ppm males sacrificed at 12 months and at termination). Incidences of findings which were increased in 750 ppm males included the following (values in parenthesis are the p by Fisher's exact test for incidences in 750 ppm males as compared with their controls):

Males which were sacrificed at 12 months (from table 27)

	0 ppm	25 ppm	250 ppm	750 ppm
liver				
diffusely dark	0/17	0/19	0/19	2/19 (0.2714)

Males dying on test or which were sacrificed moribund (from table 29):

	0 ppm	25 ppm	250 ppm	750 ppm
kidneys:				
irregularly-shaped	3/30	1/25	4/27	13/31 (0.0047)
large	0/30	0/25	0/27	2/31 (0.2541)
liver				
diffusely dark	1/30	0/25	0/27	12/31 (0.0007)
heart				
light focus(I) /area(s)	0/30	0/25	0/27	5/31 (0.0286)
perineum/perianal				
stains	8/30	9/25	9/27	15/31 (0.0683)

Males sacrificed at termination (week 66 for 750-ppm males; at week 78 for other groups); from table 28:

	0 ppm	25 ppm	250 ppm	750 ppm
liver				
diffusely dark	0/23	0/26	0/24	2/20 (0.2104)

There were no findings in females with incidences appearing to correlate with 2,4-DB dosage.

c. Microscopic pathology

1) Non-neoplastic

Among 750 ppm males, there were higher incidences of amyloidosis of a number of organs:
(values in parenthesis are the p by Fisher's exact test for incidences in 750 ppm males as compared with their controls):

Males which were sacrificed at 12 months (from table 30):

organ	0 ppm	25 ppm	250 ppm	750 ppm
kidney	5/17	9/19	8/19	10/19 (0.1418)
liver	4/17	5/19	5/19	10/19 (0.0734)
heart	2/17	n.e.	n.e.	11/19 (0.0483)
spleen	0/17	0/1	n.e.	5/18 (0.0264)
thyroid	2/17	n.e.	n.e.	11/19 (0.0483)
adrenals	5/17	n.e.	n.e.	11/19 (0.0832)
stomach	0/17	0/2	n.e.	4/19 (0.0658)
testes	4/17	n.e.	n.e.	10/19 (0.0734)

n.e. = none examined

Males dying on test or which were sacrificed moribund (from table 32):

	0 ppm	25 ppm	250 ppm	750 ppm
liver	10/30	13/25	18/26	21/31 (0.0719)
heart	10/30	12/25	18/27	21/31 (0.0719)
parathyroids	1/17	0/18	0/20	4/21 (0.2432)
adrenals	19/30	19/25	21/27	26/31 (0.0622)
stomach	4/30	1/24	6/26	10/31 (0.0723)

Males sacrificed at termination (week 66 for 750-ppm males; at week 78 for other groups); from table 31:

	0 ppm	25 ppm	250 ppm	750 ppm
thyroid	3/23	n.e.	n.e.	7/20 (0.0903)
stomach	1/23	n.e.	n.e.	4/20 (0.1319)
lacrimal gland	0/23	n.e.	n.e.	3/20 (0.0924)
testes	3/23	0/3	0/1	7/20 (0.0903)

n.e. = none examined

Other than amyloidosis of one organ or another, the only dose-related finding was hepatocellular enlargement in males sacrificed at termination:

	0 ppm	25 ppm	250 ppm	750 ppm
hepatocellular enlargement	1/23	1/26	3/24	6/20 (0.0301)

At terminal sacrifice there were "equivocal" increases in the incidence of amyloidosis in liver, spleen, kidneys, thyroid, adrenals and small intestines of female mice; from table 31 (values in parenthesis are the p by Fisher's exact test for incidences in 750 ppm females as compared with their controls):

Organ with amyloidosis	0 ppm	25 ppm	250 ppm	750 ppm
liver	0/39	1/34	0/37	3/37 (0.1105)
spleen	0/38	0/2	0/5	2/37 (0.24)
kidneys	1/39	1/34	4/37	5/37 (0.0839)
thyroid	0/39	n.e.	n.e.	3/36 (0.1057)
adrenals	0/39	n.e.	0/1	3/37 (0.1105)
ileum	11/39	n.e.	0/1	16/37 (0.1293)

n.e. = none examined

2) Neoplastic:

The following incidences are reported (vol. I, p. 30) for alveolar/bronchiolar adenomas and combined adenomas and carcinomas (p values by Fisher's exact test are given in parenthesis where these incidences were noticeably - sometimes significantly - elevated with respect to control levels):

Dose (ppm)	Total Number of mice		Number of Mice with alveolar/bronchiolar adenomas		Number of Mice with alveolar/bronchiolar adenomas & carcinomas	
	Male	Female	Male	Female	Male	Female
All mice:						
0	70	70	0	1	0	1
25	70	70	5 (0.029)	1	6 (0.014)	1
250	70	70	3 (0.122)	4 (0.183)	4 (0.060)	5 (0.104)
750	70	70	0	1	0	1
Sacrificed at 12 months:						
0	17	17	0	0	0	0
25	19	20	0	0	0	0
250	19	18	2 (0.271)	2 (0.257)	2 (0.271)	2 (0.257)
750	19	20	0	0	0	0

Dose (ppm)	Total Number of mice Male	Female	Number of Mice with alveolar/bronchiolar adenomas Male	Female	Number of Mice with alveolar/bronchiolar adenomas & carcinomas Male	Female
DOT or sacrificed moribund:						
0	30	13	0	0	0	0
25	25	16	2 (0.202)	0	3 (0.088)	0
250	27	11	0	0	1	0
750	31	13	0	0	0	0
Terminal sacrifice:						
0	23	39	0	1	0	1
25	26	34	3 (0.141)	1	3 (0.141)	1
250	24	37	1	2	1	3
750	20	37	0	1	0	1

Since the liver is a target organ (the highest dose level in this study was set at less than 1000 ppm on the basis of increased liver weights in a 4-week range-finding study; see vol. I, p. 14) the following incidences of hepatocellular adenomas and combined adenomas and carcinomas are noted (p values from Fisher's exact test, comparing incidences of a tumor type with control value, appear immediately below the elevated value):

	males				females			
12 month sacrifice:	0	25	250	750	0	25	250	750
Hepatocellular carcinomas	0/17	0/19	1/19	1/19	0/17	0/20	0/18	0/20
18 month sacrifice:								
Hepatocellular adenomas	2/23	2/26	3/24	1/20	0/39	0/34	0/37	0/37
Hepatocellular carcinomas	0/23	1/26	1/24	1/20	1/39	0/34	0/37	0/37
DOT or sacrifice moribund:								
Hepatocellular adenomas	2/30	0/25	0/26	0/31	0/13	0/16	0/11	0/13
Hepatocellular carcinomas	0/30	0/25	2/26 (0.211)	1/31	0/13	0/16	0/11	0/13
Overall incidences:								
Hepatocellular adenomas	4/70	2/70	3/69	1/70	0/69	0/70	0/66	0/70
Hepatocellular carcinomas	0/70	1/70	4/69 (0.058)	3/70	1/69	0/70	0/66	0/70
combined	4/70	3/70	7/69 (0.258)	4/70	1/69	0/70	0/66	0/70

Applying the Cochran-Armitage trend test to incidences of hepatocellular carcinomas in the 0, 25 and 250 ppm males gives a chi^2 value for linear trend of 5.3009, and this is significant (one sided) at $p \leq 0.05$. For all 4 male groups the chi^2 for linear trend is 2.120, and this is not significant (but males in the high-dose group were sacrificed 3 months before others).

Appendix L (volume V) states (under the heading "Historical Control Data"):

"Historical control data are available from Hazleton Laboratories America, Inc. However, the Environmental Protection Agency has taken the position that any study that is a part of the historical database, and is submitted in a final report, is subject to being audited in its entirety... Therefore, until this issue is resolved, we would prefer not to present these data at this time."

This appendix also contains material from the open literature regarding tumor incidences in CD®-1-HaM/ICR mice. It is not immediately evident as to what the relationship is between this strain and that (Crl:CD®1(CR)BR) used in this study.

D. DISCUSSION:

In evaluating the tumor incidence data for males, the fact that the 750 ppm group was terminated at 66 weeks and the others at 78 weeks has to be taken into account.

The overall incidence of combined bronchiolar adenomas and carcinomas in 25 ppm males (6/70) was significantly ($p = 0.00667$ by Fisher's exact test) higher than that of controls (0/70), but the overall incidence for 250 ppm males (4/70) was not ($p = 0.0598$), and 2 of the 4 tumors in the 250 ppm males were present in those animals sacrificed at 12 months (there were no bronchiolar tumors noted in any of the 750 ppm males sacrificed at 12 months).

There appears to be a possible dose-relationship involving hepatocellular carcinomas in males, with overall group incidences of 0/70, 1/70, 4/69 and 3/70 (keeping in mind that the last value is from the 750 ppm males, sacrificed at week 66). The p (by Fisher's exact test) associated with the overall incidence of hepatocellular carcinomas in 250 ppm males (4/69) versus controls (0/70) is 0.058, and this is a close approach to statistical significance. While these values are certainly not convincing evidence of oncogenicity (and it is also noted that the p obtained by comparing incidences of combined hepatocellular adenomas and carcinomas for 250 ppm males (7/69) and controls (4/70) is 0.2576), the situation has to be addressed. Although the laboratory has indicated a reluctance to submit historical control data on this mouse strain, some information (perhaps from a series of recent consecutive studies conducted in support of pesticide registrations) on this point is appropriate, particularly as there were no occurrences of bronchiolar adenomas and carcinomas, or of hepatocellular carcinomas (although hepatocellular adenomas were present) in the control males of this study.

It is concluded that the high incidence of mortalities which occurred starting about week 58 in 750 ppm males is conclusive evidence that these animals were dosed at (or even somewhat above) a maximally tolerated dose level.

With the females there is no evidence of any dose-related trend in either bronchiolar, liver or any other type of tumor. It is also noted that incidences of neoplasms in females in this study in all groups were quite low. However, the major question is whether in fact the females were tested at a maximally tolerated dose (MTD) or close enough to such a dose, particularly since the only significant effect at 750 ppm was an increase in mean kidney weights.

Justification should be made then that 750 ppm in females is a MTD (or is close enough to such a level). Information submitted should include data, preferably from both sexes, from the 4-week range-finding study (HLA No. 6158-102), which was used in setting the dose levels of this oncogenicity study.

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